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Adult Recipients of Umbilical Cord Blood Transplants after Nonmyeloablative Preparative Regimens

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ABSTRACT

We report the outcome of 13 patients with advanced malignancies who underwent nonmyeloablative conditioning therapy followed by infusion of partially matched unrelated cord blood cells. The median age of these patients was 49 years, and their median weight was 65.7 kg. The median nucleated cell dose infused was 2.07 × 10^7/kg. Eight of the 13 patients demonstrated donor chimerism between 4 weeks and 6 months, and subsequent conversion to full donor chimerism was achieved in 5 patients. Three patients were alive and free of disease at 158 to 1054 days, with a median survival of 288 days after transplantation. The 100-day event-free survival is 69%, and overall survival is 77%. At 1 year, the event-free and overall survival was 43%. Treatment-related mortality observed within the first 100 days after transplantation was low: 1 previously extensively pretreated patient died of multiorgan failure. This result provides a basis for further exploring this potentially curative approach to selected patients who lack matched related or unrelated hematopoietic stem cell donors.

KEY WORDS

Nonmyeloablative conditioning • Unrelated cord blood • Donor chimerism • Survival

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) as a form of potentially curative immunotherapy for patients with malignancies has been limited by the toxicity of myeloablative preparative regimens, the lack of matched sibling donors, and graft-versus-host disease (GVHD). Umbilical cord blood (UCB) transplantation has demonstrated promising results in both pediatric and adult patients, thereby broadening the scope of patients who may benefit from allogeneic HSCT [1-3]. However, the results of UCB transplantation involved the use of myeloablative preparative regimens that are associated with considerable morbidity and mortality and a significant delay in immune recovery. Moreover, the disease of the poor-risk patients entered into these early trials has led to disappointing outcomes for most adult patients. The conclusion that can be drawn thus far from these ablative preparatory regimens, considering that most donor-recipient pairs were HLA-disparate, is that UCB contains a sufficient number of hematopoietic stem cells (HSC) to achieve engraftment in some adult patients with a lower-than-anticipated risk of severe acute GVHD. The use of UCB as a source of stem cells allows allografting to be offered to more patients, many of whom do not have a matched sibling or unrelated donor, thus making allogeneic therapy the only chance to cure the underlying disease. With the profound influence of the UCB cell dose (both nucleated cell dose and $CD34^+$ cell dose) on engraftment, survival, and treatment-related mortality in the adult setting, we have focused on methods to allow for allogeneic engraftment with less treatment-related mortality.

Recently, older recipients of allogeneic HSCT have been treated successfully with a variety of less intense nonmyeloablative conditioning regimens [4-6]. Given the excellent tolerability of these nonmyeloablative regimens and the high rate of engraftment, there has been interest in this transplantation strategy with UCB as a source of HSC support after nonmyeloablative preparative regimens.

We and others have previously reported the re-

sults of adult patients with advanced malignancies who successfully underwent nonmyeloablative conditioning therapy followed by infusion of partially matched unrelated-donor cord blood cells [7-11]. In this article we report the clinical outcome of a larger group of 13 patients with advanced malignancies treated with this approach. In contrast to the data presented by Barker et al. [11], all these patients received a single unit of unrelated donor cord blood cells.

MATERIALS AND METHODS

Patient Eligibility

This pilot study was approved by the Institutional Review Board of Duke University Medical Center. Patients were eligible if they had a condition appropriate for allogeneic hematopoietic progenitor cell transplantation and did not have a 6/6 or 5/6 related donor or a 6/6 matched unrelated donor. All patients signed an approved consent form.

Donor Selection

Cord blood searches were performed through the Placental Blood Program at the New York Blood Center, the National Marrow Donor Program, and the Cord Blood Transplantation Study cord blood banks. Units were chosen according to degree of HLA matching and cell dose. HLA class I antigens were identified according to serologic or low-resolution DNA typing, and class II antigens were identified according to high-resolution typing of DRB1. Genetic identity at DRB1 took precedence over class I identity. If more than 1 similarly matched unit was available, then the unit with the highest cell dose was used. Units were scored for matching at class I A and B antigens and class II DRB1 alleles. All units contained a minimum cryopreserved nucleated cell dose of 1×10^7/kg recipient weight.

Preparative Regimen and Transplantation

All patients received fludarabine 30 mg/m^2 and cyclophosphamide 500 mg/m^2 daily for 4 days (days -5 to -2) with horse antithymocyte globulin 30 mg/kg/d for 3 days (days -3 to -1). After the first 10 patients, 200 cGy of total body irradiation was added to the regimen on day -1. The cryopreserved cord blood units were transported to the transplant center in a dry shipper and stored in the vapor phase of liquid nitrogen. The units were thawed in the laboratory and washed with 10% dextran and 5% human albumin before infusion as previously described [12]. Cell counts, viability, and $CD3^+$ and $CD34^+$ cell counts were determined at the time of thawing. A single unit of cord blood was infused through a central line at 1 to 3 mL/min on day 0.

GVHD Prophylaxis and Supportive Care

Acute GVHD prophylaxis consisted of cyclosporin A and methylprednisolone for the first 8 patients; the next 5 were given cyclosporine and mycophenolate mofetil. The methylprednisolone was given at 0.5 mg/kg/d on days 0 through +4 and 1 mg/kg/d on days +5 through +20, followed by a daily dose tapered by approximately 0.2 mg/kg/wk thereafter. Mycophenolate mofetil was given at 1 g twice daily orally. Cyclosporine, steroids, or mycophenolate mofetil was tapered over several months starting at day +180 in patients without evidence of chronic GVHD.

Acute and chronic GVHD were diagnosed and graded by standard criteria. Grade II to IV acute GVHD was initially treated with high-dose steroids followed by other agents at the discretion of the treating physician. Granulocyte colony-stimulating factor was administered at 5 μg/kg/d until the absolute neutrophil count was >1000/μL for 3 consecutive days. A single patient received granulocyte-macrophage colony-stimulating factor because of a previous allergic reaction to granulocyte colony-stimulating factor. Platelets were transfused for counts <10000/μL or bleeding, and packed red blood cells were administered for hematocrit <30%. Neutropenic fever was treated with broad-spectrum antibiotics. A weekly test for cytomegalovirus DNA was obtained through day +100, and patients with positive results were treated with ganciclovir (5 mg/kg twice daily for 2 weeks followed by 5 mg/kg daily Monday through Friday for 10 weeks).

Statistical Analysis

A total of 13 sequential patients were included in the analysis. We estimated overall and event-free survival through March 1, 2004, according to the Kaplan-Meier product-limit method. We calculated event-free survival as the time from transplantation to disease progression, graft failure (primary or secondary), autologous bone marrow recovery, or death from any cause, whichever occurred first. Patients who were alive and disease free were censored at the date of last follow-up visit. Overall survival was calculated from the time of transplantation to death, and patients who were alive were censored at the date of last follow-up visit.

RESULTS

Patient Characteristics

Thirteen patients underwent transplantation between May 2000 and October 2003. The patient characteristics are described in Table 1. The median age of these patients was 49 years (range, 19-62 years), and

Table 1. *Characteristics of Patients, Grafts, and GVHD Prophylaxis*

Patient No.	Age (y)/ Sex	Weight (kg)	Disease	Previous Autograft/ No. Prior Regimens	Status at Transplantation	HLA Disparity*	CMV Status	GVHD Prophylaxis	UCB NC (10^7/kg)	UCB CD34 (10^6/kg)	UCB CD3 (10^6/kg)	UCB CFU (10^4/kg)
1	62/M	65.3	MCL-REL1	No/3	Refractory	4/6 (A, B)	+	CSA/P	5.53	0.40	6.98	6.13
2	41/M	66.6	MCL-REF	Yes/3	Refractory	4/6 (A, DRB1)	+	CSA/P	2.12	0.08	4.24	NA
3	42/M	89.0	DLCL-REF	Yes/4	Refractory	4/6 (A, DRB1)	+	CSA/P	2.47	0.96	4.95	22.2
4	54/F	59.3	MDS-AML	No/0	Untreated	4/6 (A, DRB1)	−	CSA/P	1.83	0.05	2.99	2.54
5	37/F	49.1	Melanoma-REF	No/2	Refractory	4/6 (A, DRB1)	−	CSA/P	3.22	0.20	9.29	5.14
6	42/F	88.7	MDS-REF	No/2	Refractory	4/6 (A, B)	+	CSA/P	1.97	0.06	3.23	0.56
7	19/M	61.2	T-ALL-REF	No/2	Refractory	4/6 (B, DRB1)	+	CSA/P	1.07	0.44	22.82	NA
8	51/M	53.8	AML-CR3	Yes/4	CR3	5/6 (B)	+	CSA/P	2.86	0.18	6.54	9.79
9	63/F	78.0	ALL-CR1	No/1	CR1	5/6 (B)	−	CSA/MMF	2.10	0.08	2.02	3.85
10	60/F	98.7	CML-CP	No/1	CP	4/6 (A, B)	−	CSA/MMF	2.02	0.05	2.75	NA
11	43/F	63.4	T-ALL-CR3	Yes/3	CR3	5/6 (A)	+	CSA/MMF	1.87	0.04	2.65	8.6
12	48/F	75.5	Follicular NHL-REF	No/7	Refractory	4/6 (A, B)	+	CSA/MMF	2.03	0.08	3.05	6.6
13	33/F	42.0	Hodgkins-REF	Yes/5	Refractory	4/6 (A, B)	+	CSA/MMF	3.91	0.14	3.54	10.7

M indicates male; F, female; MCI, mantle cell lymphoma; REL, relapse; REF, refractory; CMV status, patients' cytomegalovirus status by serology; UCB, umbilical cord blood; NC, nucleated cell; GVHD, graft-versus-host disease; CSA, cyclosporin A; P, prednisolone; CFU, colony-forming unit; NA, not available; MDS, myelodysplastic syndrome DLCL, diffuse large-cell lymphoma; T-ALL, T-cell acute lymphoblastic leukemia; CR, complete remission; CP, chronic phase; MMF, mycophenolate mofetil; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; NHL, non-Hodgkin lymphoma.

*HLA antigen disparities between donor and recipient are shown in parentheses.

their median weight was 65.7 kg (range, 42-99 kg). The diagnoses included relapsed acute lymphoblastic leukemia (n = 3), myelodysplasia (n = 2), relapsed mantle cell lymphoma (n = 2), metastatic melanoma (n = 1), relapsed high-grade lymphoma (n = 1), relapsed acute myeloid leukemia (n = 1), relapsed Hodgkin disease (n = 1), relapsed low-grade lymphoma (n = 1), and chronic myeloid leukemia (n = 1).

Graft Characteristics

Details of HLA matching, donor and recipient sex matching, and cell doses are described in Table 1. No donor/recipient pairs were mismatched at both DR loci. The median number of nucleated cells infused per kilogram of recipient weight was 2.07×10^7 (range, 1.07- 5.53×10^7). After thawing, the median number of $CD3^+$ cells infused was 4.60×10^6/kg (range, 2.02- 22.82×10^6/kg), the median number of colony-forming units was 5.14×10^4/kg (range, 0.56- 22.2×10^4/kg), and the median number of $CD34^+$ cells was 1.3×10^5/kg (range, 0.5-9.6×10^5/kg).

Engraftment and Chimerism

The clinical outcome is summarized in Table 2. Eight of the 12 evaluable patients demonstrated donor chimerism between 4 weeks and 6 months on micro-

Table 2. *Outcome of 13 Patients after Nonmyeloablative Unrelated Umbilical Cord Blood Transplantation*

Patient No.	Donor Engraftment	Days to Neutrophils >0.5 × 10^9/L	Days to Platelets >20 × 10^9/L	Acute GVHD (Grade/Site)	Chronic GVHD (site)	Current Status	Cause of Death
1	100%	12	61	0	Yes (bronchiolitis obliterans, gut)	Dead, day 384	Cerebral infarct, no lymphoma
2	100%	11	Never*	0	No	Alive in CR, day 1054+	—
3	No	Never*	Never*	—	—	Dead, day 185	Disease progression
4	3%	13	Never*	—	—	Dead, day 680	Disease progression
5	100%	32	37	III/Gut	No	Dead, day 134	Disseminated aspergillosis
6	3.6%	6	Never*	—	—	Dead, day 193	Disease progression
7	No	Never*	Never*	—	—	Dead, day 288	Disease progression
8	No	6	6	—	—	Alive in CR, day 518+	—
9	No	Never*	Never*	—	—	Dead, day 315	Disease progression
10	3%	Never*	Never*	—	—	Dead, day 360	Disease progression
11	100%	24	26	0	No	Dead, day 33	Sepsis
12	100%	10	14	I/Gut	No	Alive in CR, 158+	—
13	NE	—	—	—	—	Dead, day 2	Multiorgan failure

GVHD indicates graft-versus-host disease; CR, complete remission; NE, not evaluable.

**Never* indicates neutrophil never <0.5 × 10^9/L or platelets never <20 × 10^9/L.

satellite polymorphism assay or fluorescent in situ hybridization for sex-mismatched donor/recipient pairs. Microsatellite polymorphisms were performed for both myeloid and lymphoid cells (CD3 selection), and in all cases the percentage of myeloid and lymphoid donor cells was similar. Five patients achieved full donor chimerism; 3 patients had a transient, low level of donor cells, but these cells did not persist. The remaining 5 patients never demonstrated any evidence of donor engraftment. One patient with refractory Hodgkin disease died on day +2 of multiorgan failure. Of note, 4 of the first 5 patients demonstrated engraftment, but several of the following patients did not. Because of this, 200 cGy of TBI was added to the regimen, and 2 of the next 3 patients engrafted; the third patient died of multiorgan failure too early to evaluate. For those who engrafted, the median time to neutrophils >500/μL was 12 days (range, 6-34 days). Four patients never experienced a decrease to <500/μL. The median time to platelets >20000/μL was 14 days (range, 6-61 days), and 7 patients did not experience decreases below this number.

Event-Free and Overall Survival

With a median follow-up of 20 months, the median event-free survival of the entire group was 288 days, with a range of 10 to 1054 days. The 100-day event-free survival was 69%, and overall survival was 77%. One-year event-free and overall survival was 43%. Three patients were alive and disease free with a median follow-up of approximately 2 years. The estimated event-free survival is 24% and overall survival is 22% at 4 years.

Graft-versus-Host Disease

Of the 8 patients with engraftment, or of the 5 patients with stable engraftment, GVHD occurred in 2 patients. In 1 patient, GVHD contributed to the development of *Aspergillus* pneumonia, and in the second patient, GVHD resolved with steroid treatment. One patient developed chronic GVHD that was controlled, but that patient died of complications of a stroke without evidence of disease at autopsy.

Toxicity and Causes of Death

Despite extensive prior therapy in most of these patients, the transplantation was well tolerated, and all patients were treated in the outpatient setting (after receiving the chemotherapy as inpatients). No patients experienced any unusual or unexpected toxicity. Two patients died of complications directly related to this procedure: 1, who had 7 prior regimens for refractory Hodgkin disease, of multiorgan failure, and another from overwhelming gram-negative sepsis when she was fully recovered hematologically and ready for discharge home. Mild vomiting occurred in 2 patients, diarrhea in 1 patient, herpes simplex virus stomatitis in 2 patients, varicella zoster virus infections in 1 patient, steroid-induced hyperglycemia in 2 patients, and cyclosporine-related toxicities in 2 patients. One patient developed transient cardiac failure that resolved over the course of several weeks with medical management. Complications related to transient cytopenia were uncommon: 2 had febrile neutropenia, and 1 had grade 3 hemorrhagic cystitis. Preemptive ganciclovir was started for cytomegalovirus DNA positivity in patients, but 2 (11%) developed visceral organ disease (1 colitis and 1 esophagitis). Disseminated *Aspergillus* infection occurred in 2 patients with GVHD 6 and 12 months after transplantation, and this was fatal in 1 patient. The primary cause of mortality was progression of the underlying disease in patients who did not engraft. It is important to note that in patients who did not experience donor cell engraftment, autologous recovery was prompt, and there were no significant sequelae from this preparatory regimen, except for the 1 patient who died on day +2.

DISCUSSION

Unrelated UCB transplantation has recently been explored in an increasing number of adult patients [12-23]. The relative ease of procurement and the lower-than-anticipated risk of severe acute GVHD has made UCB transplantation an appealing alternative to bone marrow–derived HSCs. UCB contained a sufficient number of HSCs to achieve engraftment in adult patients, with a lower-than-anticipated risk of severe acute GVHD, even when HLA-disparate grafts were infused. The use of UCB as a source of stem cells allows allografting to be offered to more patients, many of whom do not have a matched sibling or unrelated donor to allow allogeneic therapy as the only chance to cure the underlying disease. The results thus far suggest that unrelated donor UCB transplantation can result in long-term disease-free survival in some of these patients. Similar to the pediatric series, clinical experience in the adult patients has also documented the importance of graft cell dose in determining engraftment and survival. It is hoped that the advantage of a lower GVHD rate without any apparent increase in relapse in UCB transplantation will offset any adverse effect of reduced cell dose on survival. However, treatment-related mortality remains the main obstacle for successful UCB transplantation in adults. This treatment-related mortality is further enhanced by the advanced disease stages of these patients so that ultimately overall survival becomes compromised [24]. Moreover, the prolonged pancytopenia with delays in neutrophil and platelet engraftment after an ablative regimen results in a marked increase in resources as patients are hospitalized for many weeks awaiting engraftment.

One possible method to improve on the treatment-related mortality is the use of nonmyeloablative stem cell transplantation (NST) or transplantation with a reduced-intensity conditioning regimen. Use of this approach was initially based on the rationale that the therapeutic benefit of an allogeneic transplantation is partially related to the crucial immune-mediated graft-versus-malignancy effect. The NST, with its low-dose preparative regimens, makes allogeneic transplantation applicable to patients with relative contraindications to myeloablative regimens. This dose-reduced conditioning nourished the hope that patients would experience less transplant-related mortality with fewer infections and less GVHD. This approach was based on the hypothesis that the attenuated conditioning regimens would (1) decrease the mucosal and tissue damage, (2) minimize the release of inflammatory cytokines, (3) decease the incidence of infections, (4) reduce the incidence of GVHD, and (5) ultimately allow powerful alloimmune responses to eradicate disease processes while minimizing the initial treatment-related morbidity and mortality. Given the excellent tolerance of these nonmyeloablative regimens and the high rate of alloengraftment, there has been considerable interest in these transplantations strategies with UCB as a source of HSC support. NST with UCB provides an opportunity for immunotherapy for older patients, sicker patients, and patients without suitable donors, who are not eligible for this potentially curative approach.

However, there is increased concern about graft rejection with this approach. In NST with adult related or unrelated donor stem cells, the infusion of cytokine-mobilized peripheral blood progenitor cells with higher cell doses may overcome the rejection potential, with resultant stable donor cell recovery over the autologous recovery [25,26]. This may not be feasible in the setting of UCB transplantation with nonmyeloablative conditioning therapy, because there are on average 2 logs fewer cells infused than would be considered standard for matched sibling or unrelated donor transplantation. In addition to the lower cell dose, there is the added complication of HLA mismatching, which increases the risk of graft rejection. The clinical outcome with this novel approach for 2 patients with malignant lymphoma has been previously published [7]. By 3 months, both patients had 100% donor engraftment and remained in remission 6 and 12 months after transplantation. This favorable outcome demonstrated the feasibility of mismatched unrelated UCB cells, even with the nonmyeloablative preparative regimens. This report updates the outcome and the results in 13 consecutive patients.

The data presented herein demonstrate clearly that engraftment can be achieved. Although engraftment is not universal, it is important to note that the preparatory regimen is truly nonmyeloablative and not a reduced-intensity regimen. Patients who did not engraft promptly recovered their autologous cells. With the exception of the 1 patient who died on the second day after transplantation, all patients recovered their peripheral blood counts either from autologous recovery or donor-derived hematopoiesis. It is possible that increasing the preparatory regimen with a higher chemotherapy dose could lead to more stable and sustained engraftment. It is surprising that engraftment occurs at all, given that the mononuclear or stem cell dose is quite limited. In addition, most of the HLA matches were at a 4/6 level, with low-resolution typing for HLA-A and -B and high-resolution typing for HLA-DRB1. Thus, it is very likely that the matching is even less ideal than the 4/6 match if high-resolution molecular HLA-A and -B allele typing is performed. Alternatively, it is possible that engraftment occurred specifically because of the heavy pretreatment with chemotherapy in most of the patients. With the small numbers of patients, it is not possible to correlate the level of engraftment with the amount of previous chemotherapy.

Although the overall results could certainly be improved, we are encouraged by these preliminary data. The favorable overall and disease-free survival in our small series is encouraging given the fact our patients represent a poor prognostic group with relapsed or chemoresistant malignancies for whom no other source of stem cells was available for the allogeneic transplantation. A review of Table 1 demonstrates the markedly advanced stage of disease for most of the patients, and 8 of 13 patients had refractory disease. Because this was a phase I trial for engraftment only, improving on the types of patients with less advanced disease will likely improve on the overall outcome as well. It is likely that the lack of engraftment in those patients with refractory disease was a consequence of involvement of the bone marrow with the underlying disease. The resource utilization was lower given that most of these patients were treated as outpatients and that their period of neutropenia and thrombocytopenia was similar to that for other NST recipients of matched sibling or matched unrelated donors and was significantly shorter than that reported for recipients of UCB receiving an ablative regimen. Moreover, patients who did not engraft had prompt recovery of their autologous stem cells. Thus, the overall treatment-related mortality for these patients was quite low.

Immune recovery after the nonmyeloablative regimen was also analyzed, and this has been previously described [8,27]. In brief, as compared with adult recipients of UCB after a myeloablative regimen, the nonmyeloablative UCB transplant recipients had (1) earlier recovery of $CD3^+$ T cells, (2) more naive ($CD45RA^+$) T cells, (3) a more diverse and robust T-cell repertoire, and (4) earlier detectable T-cell

receptor excision circle–positive T-cells. The numbers of patients who are fully engrafted at 6 months to 1 year are still limited, and therefore further analyses of immune recovery will need to await continued patient accrual to this approach.

Recently, allogeneic unrelated UCB transplantation after a reduced-intensity conditioning regimen has been reported by several groups [9-11,13]. In the largest series, by Barker et al. [11], 43 adult patients with high-risk or advanced hematologic malignancies were given single- or double-unit UCB infusion after conditioning with 2 different fludarabine-based regimens. Engraftment exceeded 70%, and the incidence of grade III to IV acute GVHD was low, at 9%, despite the use of 1 or 2 HLA antigen–mismatched grafts in 93% of the patients. The 1-year disease-free survival of these high-risk subjects was also favorable (24%-41%). Similar to the series of Barker et al., our series consisted of a group of high-risk patients who either had extensive prior therapy (had failure with least 2 lines of chemotherapy or had autologous transplantation) or had poor-risk diseases in which there were limited curative options other than allogeneic HSCT.

The results observed in this small series suggest that UCB transplantation with a nonmyeloablative conditioning regimen is well tolerated and is associated with low early transplant-related mortality. The favorable results of T-cell recovery after such a procedure suggest that it may be possible to have an excellent outcome with such an approach. The future challenge will be to develop strategies to optimize the chance of early and durable engraftment to allow further studies of immune reconstitution and other manipulations to decrease relapse.

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